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16. ABSTRACT

The respiratory system under normal conditions has the ability to maintain sterility of the deep lung even when large numbers of microbes are deposited under laboratory conditions. A number of mechanisms have been shown to be responsible for this defense of the lung, namely, mechanical clearance via the mucociliary escalator, biological clearance mediated through the alveolar macrophage (AM), and associated cellular and humoral immunological events. The adverse effects of numerous gaseous pollutants on these pulmonary defense mechanisms against infectious disease have long been recognized. Recently, researchers have begun to investigate the propensity of trace metals for causing similar effects. This paper focuses on recent and ongoing studies in the Health Effects Research Laboratory on the toxicity of metals for these host defense mechanisms.

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JOURNAL ARTICLE

EFFECTS OF METALS ON PULMONARY DEFENSE MECHANISMS AGAINST INFECTIOUS DISEASE

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INTRODUCTION

The respiratory system under normal conditions has the ability to maintain sterility of the deep lung even when large numbers of microbes are deposited under laboratory conditions. A number of mechanisms have been shown to be responsible for this defense of the lung, namely, mechanical clearance via the mucociliary escalator, biological clearance mediated through the alveolar macrophage (AM), and associated cellular and humoral immunological events.

The adverse effects of numerous gaseous pollutants on these pulmonary defense mechanisms against infectious disease have long been recognized and have been reviewed by Coffin and Gardner (1972) and Gardner and Graham (1977). Recently, researchers have begun to investigate the propensity of trace metals for causing similar effects. This paper, while including the relevant work of other researchers, will focus on the recent and ongoing studies in our laboratory on the toxicity of metals for these host defense mechanisms.

This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

TOTAL ASSAULT ON DEFENSE MECHANISMS

One of the most sensitive tools for research in this area is termed the "infectivity model." This model system measures the results of the interaction of chemical agents with aerosols of viable Streptococcus pyogenes. Numerous parameters of host response can be measured, such as mortality, in situ bacterial growth kinetics, pathology, etc. Using this system, the effects of the chloride and sulfate salts of Cd and Ni and the oxide and chloride salt of Mn have been investigated by Gardner et al. (1976) and Adkins and Gardner (1976). In these studies, mice (CD-1, female, 20-25g, Charles River) were exposed to respirable aerosols of the appropriate metal or to filtered air for 2 hours. Immediately, or 24 hours postexposure, these animals received an aerosol of Streptococci. Figure 1 presents the combined data from these studies. The response to these metallic compounds differed. Concentrations of $CdCl_2 \geq 90$ µg Cd/m^3 and 4750 µg Mn/m^3 as Mn_3O_4 significantly increased mortality ($p < .05$) when mice were exposed to the bacteria immediately after exposure. No statistically significant effects were observed with the $MnCl_2$ concentrations tested. Also, no statistically significant mortality increase was evident with Ni unless the infectious challenge occurred 24 hours postexposure to 500 µg Ni/m^3 as $NiCl_2$. The influence of the anion was also interesting. While the effect of $CdCl_2$ was little different from $CdSO_4$, the chloride form of Ni appeared to be more damaging than the corresponding sulfate. The oxide of Mn seemed to be more deleterious than the chloride form.

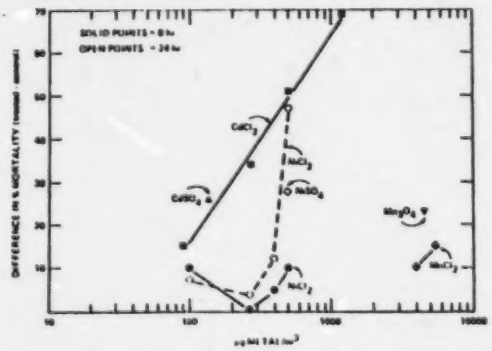

Figure 1. Percent enhancement in mortality (difference from control) on day 15 following a 2-hour exposure to aerosols of the indicated metals. The mice were challenged with viable Streptococci at 0 time (solid points) or at 24 hours (open points) following cessation of pollutant exposure. All points above 20% are significantly different from controls ($p<.05$). The only exception is $CdCl_2$ at 90 µg Cd/m^3, which was also statistically significant. (From Gardner et al., 1976; Adkins and Gardner, 1976).

In order to compare directly the toxicity of the pollutants used in these experiments, μg metal/m^3 have been converted to μg atoms metal/m^3. In this way it is possible to compare the effect of an exposure to a certain number of atoms of one metal to the same number of atoms of another metal. Therefore, for comparisons in the text, both forms of metal concentration will be used. However, since μg metal/m^3 is the more conventional expression, this form will be used on tables and graphs. When the data on Figure 1 are examined as μg atoms metal/m^3, the relative relationships remain the same. For example, the 500 μg Cd/m^3 (4.44 μg atoms Cd/m^3) exposure is similar to the 275 μg Ni/m^3 (4.68 μg atoms Ni/m^3) treatment. Conversion of all the numbers has the net effect of shifting the Ni points to the right, thereby further illustrating the greater toxicity of Cd.

Maigetter et al. (1976) have reported on the effects of a MnO_2 aerosol using a similar infectivity model. In these experiments, mice were exposed to 109 mg Mn/m^3 (as MnO_2) 3 hr/day for various lengths of time prior to a challenge with airborne Klebsiella pneumoniae or after an aerosol exposure to influenza virus. Mice exposed for 3 or 4 days and challenged with bacteria immediately after metal exposure had significantly ($p = .08$) higher mortality indices as compared to bacteria exposed controls. When influenza was used as the infectious agent 24 hours before a 3 hour exposure to MnO_2 began, there was a significant ($p < .05$) enhancement of mortality.

Using the infectivity model, prior studies by Coffin and Gardner (1972) and Gardner et al. (1976) have indicated that death is preceded by bacterial invasion of the blood which occurs following increased bacterial growth in the lungs. Furthermore, the mortality seen is thought to represent the sum of an assault on a number of defensive systems - mucociliary clearance, the AM, and the immune system.

MECHANICAL CLEARANCE

As a number of investigators have reported, the mucociliary escalator is responsible for clearing approximately 10-25% of an inhaled bacterial load (Goldstein et al., 1974; Kim et al., 1976; Fairchild and Stultz, 1976). Therefore, if this system were completely destroyed, other deep lung clearance mechanisms could still proceed, but at a decreased level.

In order to investigate the effects of pollutants on ciliary activity, in vivo and in vitro models were used by Adalis et al. (1976a, 1976b). Hamsters were exposed to respirable sized aerosols of $NiCl_2$ or $CdCl_2$ for 2 hours. Immediately, and at various days postexposure, the trachea were excised and cut into rings. The rings were cultured and ciliary beating frequency determined by synchronizing the beating with a calibrated stroboscopic microscope attachment. In the in vitro system, tracheal rings were exposed to the metals in tissue culture. Measurements were made over a 3 day period.

Figure 2 shows the results of a 2 hour exposure of hamsters to various concentrations of $CdCl_2$ or $NiCl_2$ for either 1 or 2 days. Ciliary beating frequency was determined immediately following cessation of exposure. The hamsters exposed to 275 µg Ni/m^3 (4.68 µg atoms Ni/m^3) received much more metal than those animals breathing 50 µg Cd/m^3 (0.44 µg atoms Cd/m^3), but the depression in ciliary beating was quite similar. Therefore on the basis of µg $metal/m^3$ or µg atom $metal/m^3$, Cd was more toxic than Ni. Twenty-four to 72 hour in vitro treatments to 11 µM $NiCl_2$ or 12 µM $CdCl_2$ also significantly ($p < .05$) depressed ciliary activity. Following the 24 hour incubation, Cd^{2+} was more toxic than Ni^{2+}.

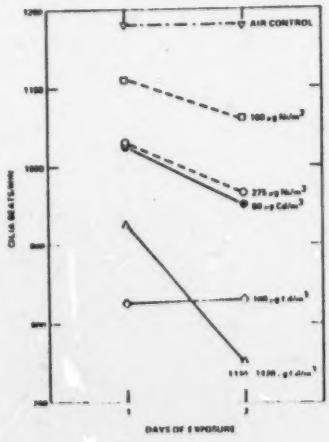

Figure 2. Beats/minute of cilia from the trachea of hamsters exposed to metal chlorides or filtered air for 2 hr/day for either 1 day or 2 days. All treatment means are significantly different from controls ($p < .05$) (From Adalis et al., 1976a, 1976b).

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When animals were allowed to recover for 24 hours following the in vivo exposure to 100 μg Ni/m^3 (2 hr/day X 2 days), significant depression in ciliary activity was still evident. Recovery had begun within 48 hours and was complete by 72 hours. Seven days following the end of a 2 day exposure to 275 μg Ni/m^3 (2 hr/day), ciliary beating frequency had not returned to control values ($p < .05$). Animals that received this pollutant for only one day exhibited normal ciliary activity at 7 days postexposure. Beating frequency of cilia from hamsters exposed to 50, 100, and 500 μg Cd/m^3 was also significantly depressed during a 48 hour recovery period.

BIOLOGICAL CLEARANCE

BACTERIAL CLEARANCE

The ability of a 2 hour exposure to $CdCl_2$ (Gardner et al., 1976) or $NiCl_2$ (Adkins and Gardner, 1976) to depress in situ clearance of viable Streptococci administered by aerosol is depicted on Figure 3. $CdCl_2$ exposed animals received the bacterial challenge immediately following pollutant exposure, while the mice exposed to $NiCl_2$ did not receive the Streptoccal aerosol until 24 hours post Ni treatment.. At the indicated time periods, the lungs of pollutant and clean air exposed mice were homogenized and viable bacterial counts determined. The microorganisms in the lungs of exposed animals grew, while those deposited in animals exposed to filtered room air exhibited a net reduction. The exposure to 500 μg Ni/m^3 (8.15 μg atoms Ni/m^3) was, on the basis of μg atoms metal/m^3, almost 3 fold higher than the aerosol of 325 μg Cd/m^3 (2.89 μg atoms Cd/m^3). This difference in concentration was not reflected in the difference between the effect of the metals on bacterial growth. Therefore while a directly determined ranking of toxicity cannot be made, the data do suggest that Cd was more potent than Ni in promoting a net increase in bacteria within the lung.

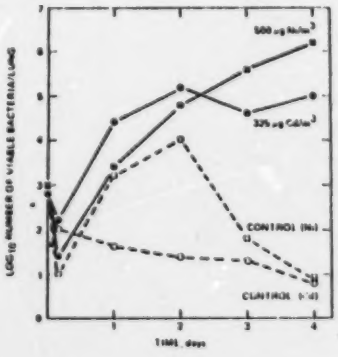

Figure 3. Log number of viable Streptococci/lung at various times following cessation of bacterial aerosol. Mice were exposed for 2 hours to aerosols of either metal chloride aerosol or filtered air immediately prior to bacterial challenge in the case of $CdCl_2$ or 24 hours prior in the case of $NiCl_2$ (From Gardner et al.,.1976;,Adkins and Gardner, 1976).

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Within the first week after the microbial aerosol, Streptococci began to appear in the blood (Gardner et al., 1976; Adkins and Gardner, 1976). Greater numbers of mice in the $CdCl_2$ and $NiCl_2$ exposure groups had bacterial contamination of their blood, as compared to corresponding control animals. A more extensive analysis of the data from $CdCl_2$ and air exposed animals showed a strong positive correlation between bacterial invasion of the blood and the mortality observed in the infectivity model.

ALVEOLAR MACROPHAGE

Investigators have attributed deep lung sterility to the proper functioning of the alveolar macrophage (Goldstein et al., 1974; Kim et al., 1976). However, the previously mentioned metals as well as other pollutants (Gardner and Graham, 1977) are capable of adversely affecting this function.

The ability of AM isolated from rats exposed for 2 hours to 500 μg Ni/m^3 (as $NiCl_2$) to phagocytize 1 μm latex spheres was determined (Adkins and Gardner, 1976). The cells harvested immediately after pollutant treatment exhibited phagocytic activity similar to cells from clean air exposed animals. However, AM lavaged 24 hours postexposure had a 19% reduction in phagocytic activity as compared to control. At this time period, a 6% reduction in ATP was observed. Interestingly, decreases in ATP, phagocytosis, and the enhancement of mortality in the infectivity model were only observed 24 hours following Ni exposure.

The total number of cells and the differential composition of cells lavaged from the lungs can also be altered by Ni or Cd treatment. Bingham et al. (1972) exposed rats for 12 hr/day, 6 days/week for 2 weeks to 109 μg Ni/m^3 (as $NiCl_2$) or 120 μg Ni/m^3 (as NiO). Rats breathing the $NiCl_2$ had an increase in total number of recoverable cells (146% of control), while animals exposed to the NiO had an even greater increase (280% of control). Adkins and Gardner (1976) exposed mice and rats to 500 μg Ni/m^3 (as $NiCl_2$) for 2 hours and found no changes in differential counts or viability of AM. However, they did find a reduction in the total number of cells recoverable from mice (62% of control) and rats (87% of control). This apparent discrepancy with the Bingham et al. (1972) study could be due to the difference in the exposure regimen.

When studying the effects of 2 hours of exposure to $CdCl_2$ at 500 and 1500 μg Cd/m^3 on rats, Gardner et al. (1976) measured similar parameters. Immediately and at 24 hours following exposure, there was a significant reduction ($p < .05$) in the viability of AM of rats exposed to the higher concentration only. Several significant differences were found when lavaged cells from exposed animals were compared to cells from clean air exposed rats. Immediately after the exposure to 1500 μg Cd/m^3, there was a reduction ($p < .05$) in the number of AM/lung (from 5.56×10^6 to 3.7×10^6), fewer total cells were recoverable, and there was an increase in polymorphonuclear leukocytes. The significant ($p < .05$) increase in total cells 24 hours postexposure to 1500 μg Cd/m^3 (from 5.54×10^6 cells/lung to 19.26×10^6 cells/lung) was primarily due to an influx ($p < .05$) of polymorphonuclear leukocytes (from 0.11×10^6/lung to 13.06×10^6/lung) even though there was also an increase ($p < .05$) in lymphocytes (from 0.08×10^6/lung to 0.83×10^6/lung). The number of AM was not different from control values 24 hours postexposure to either Cd concentration. Such changes in pulmonary cell populations could indicate inflammatory responses and possible alteration in host defense mechanisms.

The subsequent information concerning the effect of metals on AM is derived from in vitro studies. In the first series of experiments described, normal rabbit AM were allowed to attach to tissue culture bottles prior to exposure to a 0.2 μm filtrate of metal solution. Following a 20 hour incubation, the AM were harvested with trypsin and a number of parameters investigated.

The results of exposure to various concentrations of NH_4VO_3 and the chlorides of Cd^{2+}, Ni^{2+}, Mn^{2+}, and Cr^{3+} can be described in terms of the viability index (Waters et al., 1975a). This index is determined by multiplying the % viability of AM in the test culture times the ratio of the number of cells in the test culture to the number of cells in controls. This is an expression of intact viable cells remaining after exposure. The results are expressed as EC_{50} (mM concentrations of metallic ions causing a 50% reduction) (Table 1). Cadmium chloride and ammonium vanadate were most potent in reducing the viability index and specific activity of acid phosphatase. The other chlorides of Ni^{2+}, Mn^{2+}, and Cr^{3+} were less cytotoxic than Cd^{2+} and VO_3^-.

TABLE 1. EC_{50} (mM)[a] OF METALLIC IONS CAUSING DECREASE IN VIABILITY INDEX AND ENZYME ACTIVITY (FROM WATERS ET AL., 1975a)

Ion[b]	Viability Index[c]	Acid Phosphatase Specific Activity
Cd^{2+}	0.08	0.20
VO_3^-	0.10	0.09
Ni^{2+}	3.78	3.80
Mn^{2+}	4.67	5.31
Cr^{3+}	5.06	4.44

[a] mM concentration of metals causing a 50% reduction in activity following a 20 hour in vitro exposure in tissue culture bottles.

[b] Metallic chlorides were used with the exception of VO_3^-, in which case NH_4VO_3 was used.

[c] Viability Index = Viability (%) X $\frac{\text{Total intact cells in test culture}}{\text{Total intact cells in controls}}$.

Further studies of viable, metal-exposed cells indicated that phagocytosis of 1 μm latex spheres was also reduced at the following molar concentrations of metal: 2.2×10^{-5} Cd^{2+}, 3.1×10^{-3} Mn^{+2} and 5.1×10^{-4} Ni^{2+} (Graham et al., 1975a). Other relationships can also be derived from this investigation. Vanadate at 6.0×10^{-5} M caused approximately a 25% reduction in total cell numbers and a 10% reduction in viability, but had no effect on phagocytosis. Ni^{2+} at concentrations of 5.1×10^{-4} M, 7.7×10^{-4} M and 1.1×10^{-3} M, had substantial effects on phagocytosis, but had no effects on total cells remaining and minimal effects on % viability. Reductions in the phagocytic index ranged from 50 to 90%. In this system, Ni^{2+} was more toxic than Cr^{3+} or Mn^{2+}. In another investigation, using multichambered Lab-Tek slides, Waters et al. (1976b, 1976c) determined the EC_{50} for depression in phagocytic activity and ATP/mg protein following a 20 hour in vitro intubation with the chlorides of Hg^{2+}, Cd^{2+}, Zn^{2+}, Pt^{4+}, Cu^{2+}, and Ni^{2+} (Table 2). The EC_{50} of Ni^{2+} was almost 12 times higher than that of Cd^{2+} for ATP/mg protein. Cd^{2+} was also more toxic to phagocytic functioning.

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TABLE 2. EC_{50} (mM)[a] OF METALLIC CHLORIDES CAUSING DECREASED ATP AND PHAGOCYTIC ACTIVITY (FROM WATERS ET AL., 1975b, 1975c)

Metal[a]	ATP[b]	Phagocytic Activity[c]
Hg^{2+}	.04	.04
Cd^{2+}	.08	.07
Zn^{2+}	.18	.14
Pt^{4+}	.25	.21
Cu^{2+}	.37	.24
Ni^{2+}	.95	.33

[a]mM conentration of metals causing a 50% reduction in activity following a 20 hour in vitro exposure in multichamber Lab-Tek slides.

[b]ATP was measured as ATP/mg protein and calculations were based on % of control.

[c]Percent of cells phagocytizing latex spheres was calculated as % of control.

Recent studies by Hadley et al. (1976) utilized the inhibition of antibody mediated rosette formation as an index of the effect of $CdCl_2$ and $NiCl_2$ on the Fc receptor of the AM. Rosette formation by AM was determined following a 30 minute incubation with various concentrations of the metals. Both metals inhibited rosette formation in a dose dependent manner. For example, Figure 4 shows the effect of increasing concentrations of Cd on the inhibition of antibody-mediated rosette formation. Significant inhibition of rosette formation was observed with 2.2×10^{-5} M $CdCl_2$. Nickel produced significant depression only at higher concentrations (1×10^{-4} M). These data suggest that the metals may directly interfere with membrane receptors of the AM.

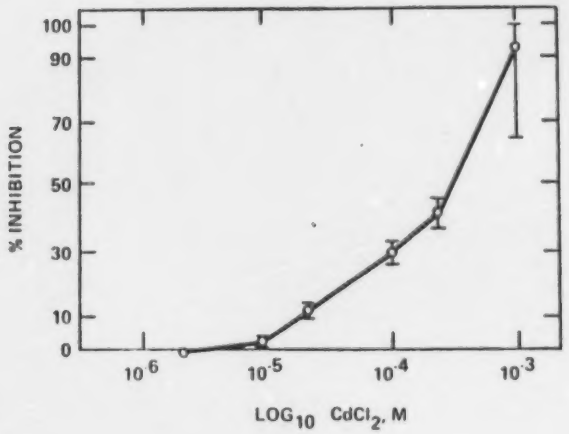

Figure 4. Percent inhibition of rosette formation by AM following a 30 minute treatment to various concentrations of $CdCl_2$. Data points are mean ± standard error (From Hadley et al., 1976).

AM have also been exposed for 20 hours to unfiltered mixtures of vanadium oxides (Waters et al., 1974). The EC_{50} for the depression in viability was found to be 0.26 mM V as V_2O_5, 0.49 mM V as V_2O_3, and 0.65 mM V as VO_2. The ranking of the EC_{50} follows the relative solubility of these compounds as evidenced by further experiments which indicated that the cytotoxicity was related to the soluble or ultrafine components (<.2μm) of the metal oxide mixture.

Since metals of environmental concern exist as complexes with different particle sizes, the influence of particle size on toxicity is an important consideration. The research of Aranyi et al. (1977) was directed to this point. Using an in vitro system, rabbit AM were exposed for 21 hours to fly ash particles coated with PbO, NiO, or MnO_2 in particle sizes of <2, 2-5, and 5-8 μm. The percentage of metal adsorbed on the fly ash particles was similar. Since the AM were exposed to concentrations of particles, the extracellular milieu contained constant amounts of metal, irrespective of particle size. As particle size decreased, more AM phagocytized the particles. It was determined that the decreases in AM viability, total protein, and LDH activity were statistically associated with decreasing particle size. This effect was due to the metal coated particles, not to solubilization of the metals in the media. Therefore the smaller the particle, the more toxic the response. Examination of EC_{75} values for viability showed that PbO coated fly ash was more toxic for each of the size ranges studied. There was little difference between NiO and MnO_2. This research demonstrates that in inhalation toxicology, it is important to consider the size of the particle delivered to the AM as well as the size range that will permit deep lung penetration.

IMMUNE SYSTEM

In view of the propensity of metals for enhancing bacterial pathogenesis, the study of these pollutants was expanded to include the humoral immune system due to its importance in host defense against many infectious agents. In these investigations (Graham et al., 1975b, 1976), mice were exposed to pollutants and immediately immunized intraperitoneally with sheep red blood cells. Four days later the number of antibody producing spleen cells was determined using the Jerne hemolytic plaque technique.

In the first series of experiments (Graham et al., 1976), animals were exposed to aerosols of $NiCl_2$ or $CdCl_2$ for 2 hours. Table 3 illustrates the results. $NiCl_2$, at concentrations $\geq$ 275 $\mu g\ Ni/m^3$, caused a statistically significant ($p < .05$) depression in the number of antibody producing spleen cells/10^6 cells. $CdCl_2$ only caused a significant suppression at 190 $\mu g\ Cd/m^3$. If the data are compared on the basis of μg atoms metal/m^3, the concentration of 110 $\mu g\ Ni/m^3$ (1.87 μg atoms Ni/m^3) is similar to 190 $\mu g\ Cd/m^3$ (1.69 μg atoms Cd/m^3). This level of Cd caused a greater depression in the number of plaque producing cells ($p < .0001$) than did Ni exposure.

TABLE 3. INFLUENCE OF METAL AEROSOLS ON NUMBER OF ANTIBODY PRODUCING SPLEEN CELLS (FROM GRAHAM ET AL., 1976)

Metal (μg Metal/m^3)	Plaques/10^6 Cells (Control - Metal Treated)
110 Cd	-95
190 Cd	280*
110 Ni	80
275 Ni	110*
380 Ni	165*
490 Ni	210*

*Significantly different from control ($p < .05$).

In another set of experiments (Graham et al., 1976) mice received $NiCl_2$, $NiSO_4$, NiO, $CdCl_2$ or $CrCl_3$ via intramuscular (IM) injection. The results for $NiCl_2$, illustrated in Figure 5, show a significant ($p < .05$) negative linear dose response relationship. The 9.26 and 12.34 µg Ni/g body weight treatment produced a statistically significant ($p < .05$) reduction in Log plaques/10^6 cells. No linear dose response relationship was found with the other metal treatments, although $NiSO_4$ at concentrations of 6.17, 9.26 and 12.34 µg Ni/g body weight did cause a significant immunosuppression when compared to treatments of 3.90 µg Ni/g or the diluent injected control group. Nickel oxide at concentrations between 3.90 to 12.31 µg Ni/g did not depress plaque formation. From these data, one would infer that $NiCl_2$ and $NiSO_4$ are more toxic than NiO.

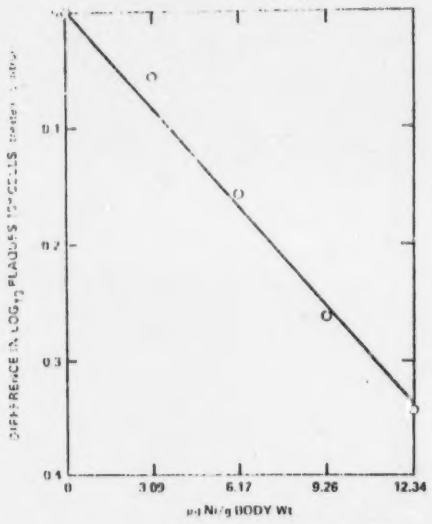

Figure 5. Difference in Log plaques/10^6 spleen cells (treated - control) on day 5 of mice immunized with sheep red blood cells and treated with IM injections of $NiCl_2$ on day 1. There was a significant linear dose response regression ($p < .05$) (From Graham et al., 1976).

Although $CdCl_2$ and $CrCl_3$ caused a slight reduction in the Log plaques/10^6 cells at the highest concentrations tested, there was no statistically significant difference following treatments of 1.1-12 µg metal/g body weight. Considering the toxicity of Cd for other systems described in this paper, this lack of immunosuppression is surprising in light of the fact that the highest concentration (11.81 µg Cd/g) resulted in 17% mortality. Ni concentrations tested in this study did not affect survival. Without additional information, it is not possible to speculate on the mechanisms.

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Some interesting observations can be made when the data from aerosol or IM treatment with $NiCl_2$ are compared. Using literature values for respiratory parameters for mice, it was calculated that the lowest effective aerosol exposure (275 µg Ni/m^3) delivered a maximum of approximately 1.94 µg Ni/mouse. The lowest effective IM dose delivered an average of 208 µg Ni/mouse. This represents an approximate 100 fold difference in the effective concentration. The greater potency of the aerosol route might reflect Ni clearance. Table 4 shows the clearance of Ni from the spleen following aerosol exposure and indicates that a significant amount of the metal is present 24 hours postexposure. When 6.17 µg Ni/g were injected IM, no Ni was detectable over this same time period.

TABLE 4. CLEARANCE OF $NiCl_2$ FROM THE SPLEEN OF MICE EXPOSED TO 625 µg Ni/m^3 FOR 2 HOURS (FROM GRAHAM ET AL., 1976)

Days Postexposure	µg Ni/g Dry Weight ($\bar{X}$ + SE)
0	1.05 ± .086
1	0.86 ± .045
2	0.78 ± .065
3	1.35 ± .190
4	1.29 ± .373

Often in making predictive judgements on pollutant toxicity, the concentrations in air and water are considered for their contribution to total body dose. In many cases, the air contribution is judged relatively insignificant when compared to the levels resulting from water or food. However, the results from these experiments seem to indicate that route of exposure could be as, or more important than absolute concentration in terms of resultant toxicity. Therefore such predictions should not ignore a relatively small aerosol dose unless there is experimental evidence to support such a contention.

SUMMARY

A number of metals are capable of reducing the effectiveness of host defense mechanisms. Most of the metals used in these in vivo investigations were used in concentrations

below the Threshold Limit Value (TLV) (American Conference of Governmental Industrial Hygienists, 1971). The TLV for these metals is as follows: 200 Cd/m^3 , 100 µg Ni/m^3, and 5000 µg Mn/m^3. In the infectivity model, Cd was more toxic than Ni. The various components of the model were affected similarly. Ciliary activity and pulmonary bacterial clearance were also more affected by Cd than by Ni.

The studies using in vitro systems with AM also show that Cd was more toxic than Ni, with EC_{50} differences of 47, 19, 12, and 5 fold for the viability index, specific activity of acid phosphatase, ATP/mg protein, and phagocytic activity, respectively. Since the AM is primarily responsible for bacterial clearance, these effects could aid in explaining the enhancement of respiratory infection found in animal models.

Studies designed to examine the systemic humoral immune system following aerosol exposures show that Cd was more immunosuppressive than Ni on a µg atom metal/m^3 basis. Concentrations of Cd aerosols which enhanced mortality in the infectivity model and decreased ciliary activity only had a slight effect on antibody production by splenic lymphocytes, but the lower (275 µg Ni/m^3) $NiCl_2$ aerosol exposure which produced immunosuppression did not increase mortality in the infectivity model. These results are not inconsistent with the mechanisms involved in the infectivity model. Most major events associated with the infectivity model, i.e. bacterial growth, invasion of the blood, and increased mortality, begin 4-5 days following bacterial challenge. A primary immune response would just be beginning at this time (Kaltreider et al., 1974), and thus for the immune system to be highly effective in the infectivity model, a localized pulmonary primary immune response or an already existing nonspecific system would be needed. The immunological model described in this report is a systemic one. Since the best immunological protection of the lung occurs when the route of immunization is also via the lung (Waldman and Ganguly, 1974), the immunological model used here is only marginally applicable to the infectivity model.

In order to directly relate the immune system to the infectivity model, it will be necessary to design experiments to test for pollutant effects on the pulmonary immune system, both as it exists as a steady state level and as it can be enhanced by immunization. The immunosuppression reported here should be interpreted in terms of a pollutant effect on a systemic host defense mechanism, a potentially hazardous event

considering the total function of the immune system. It seems possible, however, that metals capable of affecting the systemic immune system could also alter pulmonary immune functioning.

The in vitro study that was conducted on a single component of pulmonary immune mechanisms, i.e. the Fc receptor of AM, did follow the trends seen in the infectivity model. Cadmium chloride inhibited the Fc receptor more than $NiCl_2$. This would be expected to result in a decreased binding of opsonized bacteria to the AM and hence, decreased phagocytosis and increased opportunity for bacterial growth in the lung.

Given the prediliction of various forms of Cd, Ni, Mn, V, Cr, and Pb for depressing host defense mechanisms, it is important to continue toxicity studies of these compounds. It is also advisable to continue the development of more sensitive techniques to further investigate the toxic effects of these metals, as well as other materials of environmental concern. Since the model systems described in this paper have proven effective for studies of both oxidants and metals, it seems advisable to use them for other ubiquitous pollutants.

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